

**FIG.\_ 1****FIG.\_ 1A****FIG.\_ 1B****FIG.\_ 1A****SEQ ID NO: 1****Nucleotide Sequence Tankyrase Homologue isotype1**

CTTTGAAGACACTGGATTCATACTTTGCCTGGGTTATCTCTGTGTCACATAGACAAATA  
TTAGCTGTGAGCAGATCTTTGTTGCTTGTAGTCCCCAGTTAGCAGAACATTCTGTGAGA  
TAGATGTGGGAAAGGAATTCTAGCAAGAGTTGTCAGTGTATCATAAGGTGTGATTACATATTAA  
GTTTTATACTTTAACATCTGAAAATGTATACTAAATATGCAGAACTCTATTGTAGAGTGAGAAA  
CATTTGAACCTTGAGCTTCAGTCACTTATTGTATTCTTCTTGAGGTTAGCAGTAGTACCAACCCA  
AGGCAC TGCTTAGGTACCACTGCTGCTTAGTGGAGAGTCCCTCTGGCTTATCATTAGGTTGGCG  
GAAAGACGTAGTTGAATATTGCTTCAGAATGGTCAAGTGTCCAAGCACGTGATGATGGGGCCTTAT  
TCCTCTCATAATGCATGCTCTTGGTCACTGCTGAAGTAGTCATTCCCTTGCACATGGCAGA  
CCCCAATGCTCGAGATAATTGAATTATACTCCTCTCCATGAAGCTGCAATTAAAGGAAAGATTGATGT  
TTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCACCGAAATACAGATGGAAGGACAGCATTGGA  
TTTAGCAGATCCATCTGCCAAAGCAGTGCTACTGGTAATATAAGAAAGATGAACTCTTAGAAAGTGC  
CAGGAGTGGCAATGAAGAAAAATGATGGCTCTACTCACACCATTAAATGTCAGTGCACGCCAGTGA  
TGGCAGAAAGTCAACTCCATTACATTGGCAGCAGGATAAACAGAGTAAAGATTGTACAGCTGTTACT  
GCAACATGGAGCTGATGCCATGCTAAAGATAAAAGGTGATCTGGTACCATACACAATGCCCTGTTCTTA  
TGGTCATTATGAAGTACTGAACTTTGGTCAAGCATGGTGCCTGTGTAATGCAATGGACTGTGGCA  
ATTCACTCCTCTCATGAGGCAGCTCTAAGAACAGGGTTGAAGTATGTTCTCTCTTAAGTTATGG  
TGCAGACCCAACACTGCTCAATTGTCACAATAAAAGTCTATAGACTTGGCTCCCACACCACAGTTAAA  
AGAAAGATTAGCATATGAATTAAAGGCCACTCGTTGCTGCAAGCTGCACGAGAAGCTGATGTTACTCG  
AATCAAAAAACATCTCTGGAAATGGTGAATTCAAGCATCCTCAAACACATGAAACAGCATTGCA  
TTGTGCTGCTGCATCTCCATATCCAAAAGAAAGCAAATATGTGAACTGTTGCTAAGAAAAGGAGCAA  
CATCAATGAAAAGACTAAAGAATTCTTGACTCCTCTGCACGTGGCATCTGAGAAAGCTCATAATGATGT  
TGGTGAAGTAGTGGTGAACATGAAGCAAAGGTTAATGCTCTGGATAATCTGGTCAGACTCTACA



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CAGAGCTGCATATTGGTCATCTACAAACCTGCCGCTACTCCTGAGCTATGGGTGTGATCCTAACAT  
TATATCCCTCAGGGCTTACTGCTTACAGATGGAAATGAAAATGTACAGCAACTCCTCCAAGAGGG  
TATCTCATTAGGTAAATTAGGCAGACAGACAATTGCTGGAAGCTGCAAAGGCTGGAGATGTCGAAAC  
TGTAaaaaaaACTGTGTACTGTTAGGTCAACTGCAGAGACATTGAAGGGCGTCAGTCTACACCCT  
TCATTTGCAGCTGGGTATAACAGAGTGTCCGTGGAAATATGCTACAGCATGGAGCTGATGTGCA  
TGCTAAAGATAAAAGGAGGCCTTGTACCTTGACAATGCATGTTATGGACATTATGAAGTTGAGA  
ACTTCTTGTAAACATGGAGCAGTAGTTAATGTAGCTGATTTATGGAAATTACACCTTACATGAAGC  
AGCAGCAAAAGGAAATATGAAATTGCAAACCTCTGCTCCAGCATGGTGCAGACCCCTACCAAAAAAA  
CAGGGATGGAAATACTCCTTGGATCTGTTAAAGATGGAGATAAGATATTCAAGATCTGCTTAGGGG  
AGATGCAGCTTGCTAGATGCTGCCAAGAAGGGTTGTTAGCCAGAGTGAAGAAGTTGCTTCTCCTG  
TAATGTAATTGCCGCGATAACCAAGGCAGACATTCAACACCTTACATTAGCAGCTGGTTATAATAA  
TTTAGAAGTTGCAGAGTATTGTTACAACACGGAGCTGATGTGAATGCCAAGACAAAGGAGGACTTAT  
TCCTTACATAATGCAGCATCTACGGGCATGTAGATGTAGCAGCTCTACTAATAAGTATAATGCATG  
TGTCAATGCCACGGACAAATGGGCTTCACACCTTGACGAAGCAGGCCAAAGGGACGAACACAGCT  
TTGTGCTTGCTAGCCATGGAGCTGACCCACTCTAAAATCAGGAAGGACAAACACCTTAGA  
TTTAGTTTCAGCGGATGATGTCAGCGCTCTGACAGCAGCCATGCCCATCTGCTCTGCCCTTTG  
TTACAAGCCTCAAGTGTCAATGGGTGAGAAGCCCAGGAGCCACTGCAGATGCTCTCTCAGGTCC  
ATCTAGCCCATCAAGCCTTCTGCAAGCAGCAGTCTGACAACCTATCTGGGAGTTTCAGAACTGTC  
TTCAGTAGTTAGTCAAGTGGAACAGAGGGTGCTCCAGTTGGAGAAAAGGAGGTTCCAGGAGTAGA  
TTTAGCATAACTCAATTGTAAGGAATCTGGACTTGAGCACCTAATGGATATATTGAGAGAGAAC  
GATCACTTGGATGTATTAGTTGAGATGGGCACAAGGAGCTGAAGGAGATTGGAATCAATGCTTATGG  
ACATAGGCACAAACTAATTAAAGGAGTCGAGAGACTTATCTCCGGACAACAAGGTCTAACCCATATT  
AACTTTGAACACCTCTGGTAGTGGAACAAATTCTTATAGATCTGCTCTGATGATAAGAGTTCACT  
TGTGGAGGAAGAGATGCAAAGTACAGTTCAGAGACAGAGATGGAGGTCTGCAGGTGGAATCTCAA  
CAGATACAATATTCTCAAGATTAGGTTGTAACAAGAAACTATGGAAAGATAACACTCACCGGAG  
AAAAGAAGTTCTGAAGAAAACCACAACCATGCCATGAACGAATGCTATTCTGAGGTCTCCTTTGT  
GAATGCAATTATCCACAAAGGCTTGATGAAAGGCATGCGTACATAGGTGGTATGTTGGAGCTGGCAT  
TTATTTGCTGAAAACCTTCCAAAAGCAATCAATATGTATATGGAATTGGAGGAGGTACTGGGTGTC  
AGTTCACAAAGACAGATCTGTTACATTGCCACAGGCAGCTGCTCTTGCCGGTAACCTGGAAA  
GTCTTCTGCAAGTCACTGCAATGAAAATGGCACATTCTCCTCCAGGTCTACACTCAGTCAGTGGTAG  
GCCAGTGTAAATGGCCTAGCATTAGCTGAATATGTTATTACAGAGGAGAACAGGCTTACCTGAGTA  
TTAATTACTTACCAAGATTAGGGCCTGAAGGTATGGTCAAGGATAAAAGTTATTTAAGAAACTA  
ATTCCACTGAACCTAAAATCATCAAAGCAGCAGTGGCCTCTACGTTTACTCCTTGCTGAAAAAA  
AA

**FIG.-1B**

**FIG.\_2****FIG.\_2A****FIG.\_2B****FIG.\_2A****SEQ ID NO: 2****Nucleotide Sequence Tankyrase Homologue isotype2**

CGCGCTGCTCCGCCCGCCGGGGCAGCCGGGGCAGGGAGCCCAGCGAGGGCGCGTGGCGCG  
CCCATGGACTGCGCCGGATCCGGTACAGCAGGGAGCCAAGCGGCCGGGCCTGAGCGCGTCTCTC  
CGGGGGGCCTCGCCCTCCTGCTCGCGGGCCGGCTCCTGCTCCGGTTGCTGGCGCTGTTGCTGGCTG  
TGGCGGGCGGCCAGGATCATGTCGGGTGCGCCGCTGCGCCGGCGGGGAGCGGCCCTGCGCGAGCGCCGCG  
CCGAGGCCGTGGAGCCGGCCCGAGAGCTGTTGAGGCCTGCCAACGGGACGTGGAACGAGTCA  
AGAGGCTGGTGACGCCTGAGAAGGTGAACAGCCGACACGGCGGAGGAAATCCACCCGCTGCAC  
TCGCCGCAGGTTTGGCGAAAGACGTAGTTGAATATTGCTTCAGAACATGGCAAATGTCCAAGCAC  
GTGATGATGGGGCCTTATTCCCTTCATAATGCATGCTCTTGGTATGCTGAAGTAGTCAATCTCC  
TTTGCACATGGTGAGACCCAAATGCTCGAGATAATTGAAATTATACTCCTCTCCATGAAGCTGCAA  
TTAAAGGAAAGATTGATGTTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCGAAATACAG  
ATGGAAGGACAGCATTGGATTAGCAGATCCATGCCAACAGCAGTGCTTACTGGTGAATATAAGAAAG  
ATGAACCTTAGAAAGTGCCAGGAGTGGCAATGAAGAAAAATGATGGCTACTCACACCATAATG  
TCAACTGCCACGCAAGTGATGGCAGAAAGTCAACTCCATTACATTGGCAGCAGGATATAACAGAGTAA  
AGATTGTACAGCTGTTACTGCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCAT  
TACACAATGCCCTGTTCTTATGGTCAATTGAAAGTAAGTAACTGAACCTTGGTCAAGCATGGCCTGTGAA  
ATGCAATGGACTTGTGGCAATTCACTCCCTTCATGAGGCAGCTCTAAGAACAGGGTTGAAGTATGTT  
CTCTTCTCTTAAGTTATGGTGAGACCCAAACACTGCTCAATTGTCACAATAAAAGTGTATAGACTTGG  
CTCCCACACCACAGTTAAAGAAAGATTAGCATATGAATTAAAGGCCACTCGTTGCTGCAAGCTGCAC  
GAGAAGCTGATGTTACTCGAATCAAAAACATCTCTGAAATGGTGAATTCAAGCATCCTCAA  
CACATGAAACAGCATTGCATTGTGCTGCGATCTCCATATCCAAAAGAAAGCAAATATGTGAACTGT  
TGCTAAGAAAAGGAGCAAACATCAATGAAAAGACTAAAGAATTCTGACTCCTCTGACGTGGCATCTG  
AGAAAGCTCATAATGATGTTGAAGTAGTGGTAAACATGAAGCAAAGTTAATGCTCTGGATAATC

TTGGTCAGACTCTACACAGAGCTGCATATTGTGGTCATCTACAAACCTGCCGCTACTCCTGAGCT  
ATGGGTGTGATCTAACATTATATCCCTCAGGGCTTACTGCTTACAGATGGAAATGAAAATGTAC  
AGCAACTCCTCCAAGAGGGTATCTCATTAGGTAAATTAGGGCAGACAGACAATTGCTGGAAGCTGCAA  
AGGCTGGAGATGTCGAAACTGTAAAAAAACTGTGTACTGTTCAGAGTGTCAACTGCAGAGACATTGAAG  
GGCGTCAGTCTACACCACTCATTGCAGCTGGTATAACAGAGTGTCCGTGGTGAATATCTGCTAC  
AGCATGGAGCTGATGTGCATGCTAAAGATAAAGGAGGCCCTGTACCTTGACAAATGCATGTTCTTATG  
GACATTATGAAGTTGCAGAACTTCTGTTAACATGGAGCAGTAGTTAATGTAGCTGATTATGGAAAT  
TTACACCTTACATGAAGCAGCAGAAAAGGAAATATGAAATTGCAAACCTCTGCTCCAGCATGGTG  
CAGACCCTACCAAAAAACAGGGATGGAATACTCCTTGGATCTGTTAAAGATGGAGATACAGATA  
TTCAAGATCTGCTTAGGGAGATGCAGCTTGCTAGATGCTGCCAAGAAGGGTTGTTAGCCAGAGTGA  
AGAAGTGTCTTCTGATAATGTAATTGCCGCGATAACCAAGGCAGACATTCAACACACCTTACATT  
TAGCAGCTGGTTATAATAATTAGAAGTTGCAGAGTATTGTTACAACACGGAGCTGATGTGAATGCC  
AAGACAAAGGAGGACTTATTCTTACATAATGCAGCATCTACGGGATGTAGATGTAGCAGCTCTAC  
TAATAAAAGTATAATGCATGTCATGCCACGGACAAATGGCTTACACCTTGACGAAGCAGGCC  
AAAAGGGACGAACACAGCTTGTGCTTGTCTAGCCCAGGACTCTTAAAGGACT  
AAGGACAAACACCTTAGATTTAGTTAGCTCAGCGGATGATGTCAGCGCTCTGACAGCAGCCATGCC  
CATCTGCTCTGCCCTTGTACAAGCCTCAAGTGTCAATGGGTGAGAAGGCCAGGAGCCACTGCAG  
ATGCTCTCTCTTCAGGTCCATCTAGCCATCAAGCCTTCTGCAGCCAGCAGTCTGACAACATTATCTG  
GGAGTTTCAGAACTGTCTCAGTAGTTAGTTCAAGTGGAACAGAGGGTGTCCAGTTGGAGAAAA  
AGGAGGTTCCAGGAGTAGATTTAGCATAACTCAATTGTAAGGAATCTGGACTTGAGCACCTAATGG  
ATATATTGAGAGAGAACAGATCACTTGGATGTATTAGTTGAGATGGGCACAAGGAGCTGAAGGAGA  
TTGGAATCAATGCTTATGGACATAGGCACAAACTAATTAAAGGAGTCGAGAGACTTATCTCCGGACAAC  
AAGGTCTAACCCATATTAACTTGAACACCTCTGGTAGTGGAACAAATTCTTATAGATCTGCTCTG  
ATGATAAAGAGTTCAGTCTGTGGAGGAAGAGATGCAAAGTACAGTTCAGAGCAGAGATGGAGGTC  
ATGCAGGTGGAATCTCAACAGATAAAATTCTCAAGATTCAAGGTTGTAACAAGAAACTATGGG  
AAAGATAACACTACCGGAGAAAAGAAGTTCTGAAGAAAACCACAACCAGCCAAATGAACGAATGCTAT  
TTCATGGGTCTCTTGTGAATGCAATTATCCACAAAGGCTTGTGAAAGGCATGCGTACATAGGTG  
GTATGTTGGAGCTGGCATTATTGCTGAAACTCTTCCAAAAGCAATCAATATGTATATGGAATTG  
GAGGAGGTACTGGGTGTCCAGTTCAAAAGACAGATCTGTTACATTGCCACAGGCAGCTGCTCTT  
GCCGGGTAACCTGGGAAAGTCTTCCTGCAGTCAGTCAATGAAAATGGCACATTCTCCAGGTC  
ATCACTCAGTCAGTGGTAGGCCAGTGTAAATGCCCTAGCATTAGCTGAATATGTTATTACAGAGGAG  
AACAGGCTTATCCTGAGTATTAAATTACCAAGATTGAGGCCTGAAGGTATGGTCGATGGATAAA  
TAGTTATTTAAGAAACTAATTCCACTGAACCTAAATCATCAAAGCAGCAGTGGCCTTACGTTTAC  
TCCTTGCTGAAAAAA

**FIG.\_2B**

**SEQ ID NO: 3**

**Amino Acid Sequence Tankyrase Homologue isotype1**

GFGRKDVVEYLLQNGASVQARDDGGLIPLHNACSGHAEVNLLRHGADPNARDNWNYTPLHEAAIKG  
KIDVCIVLQLQHGAEPTIRNTDGRITALDPSAKAVLTGEYKKDELLESARSGNEEK**MALLT**PLNVNC  
HASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVNAM  
DLWQFTPLHEAASKNRVECSLLSYGADPTILLNCHNKSAILAPTPQLKERLAYEFKGHSLLQAAREA  
DVTRIKKHLSEMVNFKHPOQTHETALHCAAASPYPKRQICELLRKGANINEKTKEFLTPLHVASEKA  
HNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLSYGCDPNIISLQGFTALQMGNENVQQL  
LQEGLISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHG  
ADVHAKDKGGLVPLHNACSYGHYEVABELVKHGAVVNADLWKFTPLHEAAAKGKYEICKLLLQHGADP  
TKKNRDGNTPLDVKDGTDIQDLLRGDAALLDAAKGCLARVKKLSSPDNVNCRDTQGRHSTPLHLAA  
GYNNEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEAAQKG  
RTQLCALLLAHGADPTLKNQEGOTPDLVSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATADAL  
SSGPSSPSSLSAASSLDNLGSFSELSSVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLMDF  
EREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVVERLISGQQGLNPYLTNNTSGSGTILIDLSPDDK  
EFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKLWERYTHRRKEVSEENHNHANERMLFHG  
SPFVNAAIHKGFDERHAYIGGMFGAGIYFAENSSKSQNQYVYGGTGCPVHKDRSCYICHROQLLFCRV  
TLGKSFLQFSAMKMAHSPPGHHSVTGRPSV

**FIG.\_3**

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**SEQ ID NO: 4****Amino Acid Sequence Tankyrase Homologue isotype2**

RCSARRGAAGGQGAQRGARVGAAGTAPDPVTAGSQAARALSASSPGLALLLAGPGLLLRLALLAV  
AAARIMSGRRCAGGAACASAAAEEAVEPAARELFEACRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHF  
AAGFGRKDVEYLLQNGANVQARDDGGLIPLHNACSFHAEVVNLLLHGADPNARDNWNYTPLHEAAI  
KGKIDVCIVLQLQHGAEPITRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMALLTPLNV  
NCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVN  
AMDLWQFTPPLHEAASKNRVECSLLSYGADPTLLNCHNKAIDLAPTPQLKERLAYEFKGHSLLQAAR  
EADVTRIKKHLSEMVNFHPQTETALHCAAASPYPKRKQICELLRLGANINEKTKEFLTPLHVASE  
KAHNDVVEVVVKHEAKVNALDNLGQTSLLHRAAYCGHLQTCRLLSYGCDPNIISLQGFTALQMGNENVQ  
QLLQEGLSLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQ  
HGADVHAKDKGGLVPLHNACSYGHYEAELLVKHGAVVNADLWKFTPLHEAAAKGKYEICKLLLQHGA  
DPTKKNRDGNTPLDLVKDGTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRTQGRHSTPLHL  
AAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAAASYGHVDVAALLLIKYNACVNATDKWAFTPPLHEAAQ  
KGRTQLCALLLAHGADPTLKNQEGQTPLDLSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATAD  
ALSSGPSSPSSLSAASSLDNLGSFSSELSSVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLM  
IFEREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVVERLISGQQGLNPYTLNTSGSGTILIDLSPD  
DKEFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKLWERYTHRRKEVSEENHNHANERMLF  
HGSPFVNAAIHKGFDERHAYIGGMFGAGIYFAENSSKSQNQYVYGIGGGTGCPVHKDRSCYICHQOLLFC  
RVTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAELYRGEQAYPEYLITYQIMRPEGMVDG

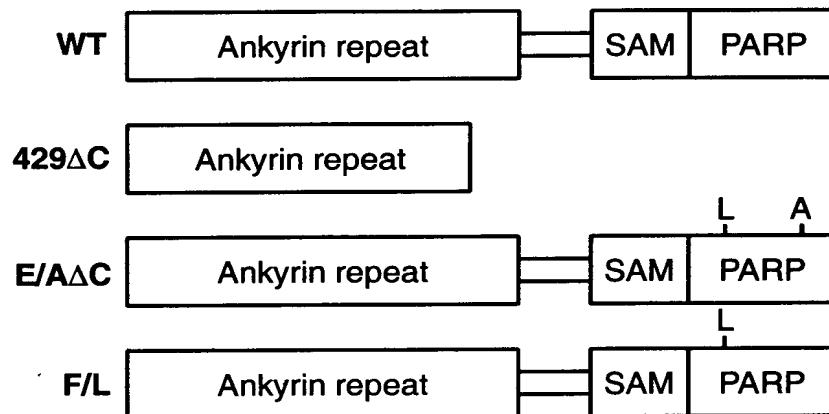
**FIG.\_4**

### Schematic Presentation of Dominant Negative Mutants for Tankyrase Homologue

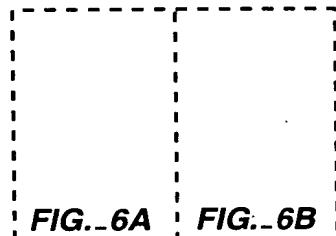
#### Dominant Negative Mutants

Truncation: 429ΔC- of the C-terminal catalytic domain – truncation of the catalytic domain of PARP acts as a dominant negative when overexpressed *in vivo* (Oncogene 1999 Nov 25; 18(50):7010-5)

Point mutant: E945AΔC- conserved residue in PARP domain, thought to be important in NAD<sup>+</sup> binding

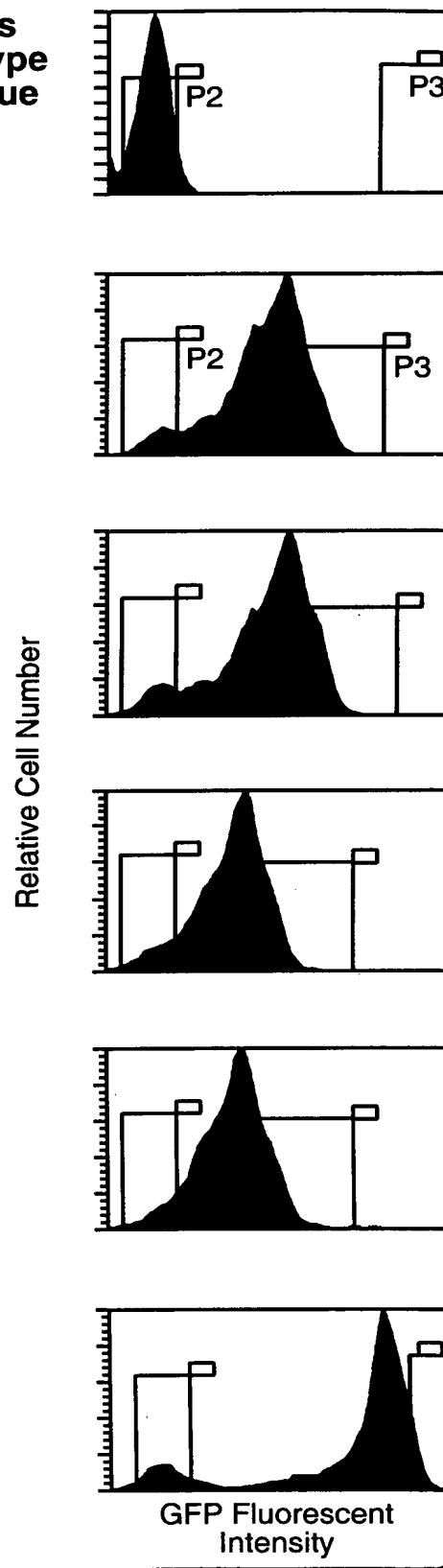


**FIG.\_5**



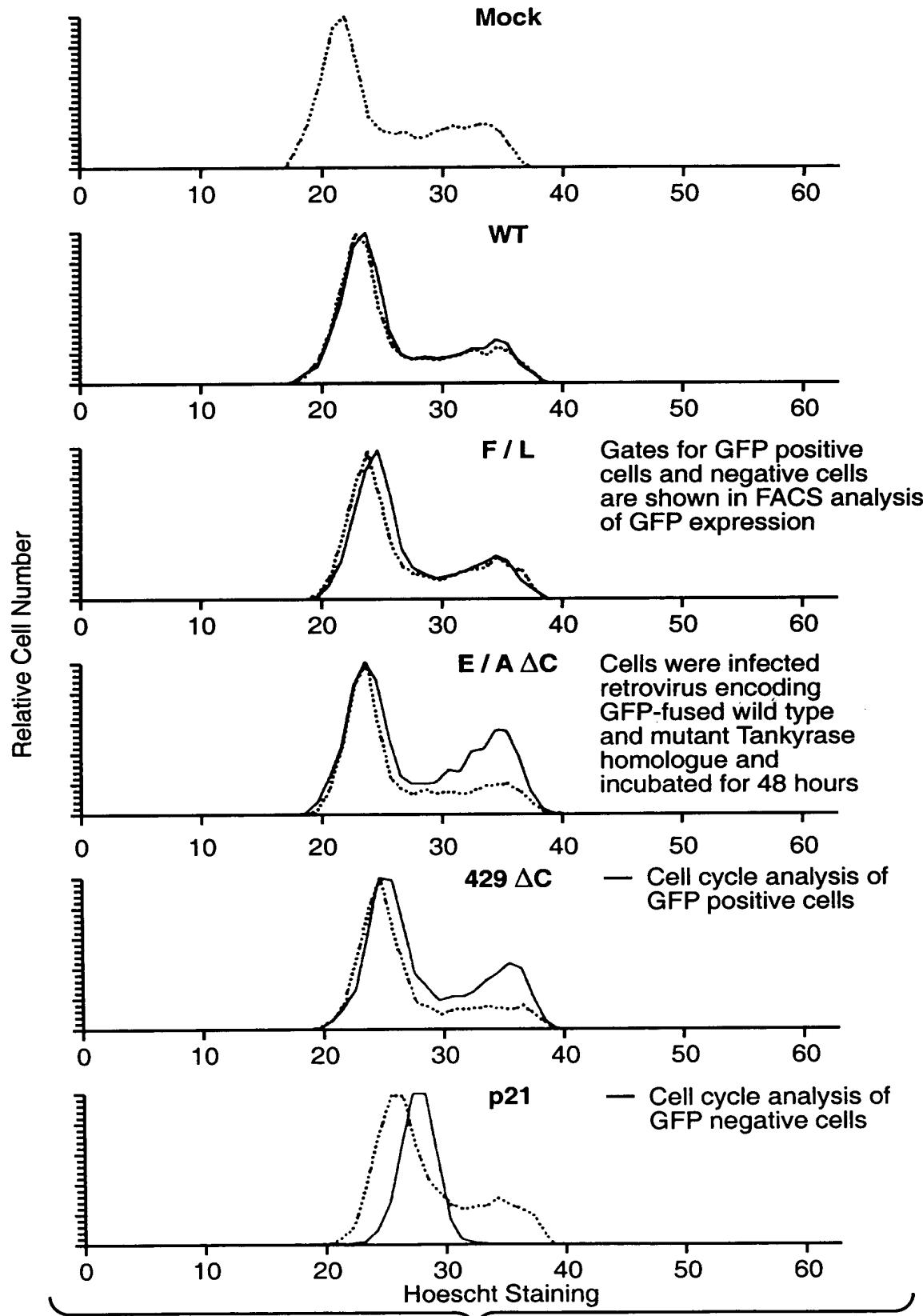
**FIG.\_6**

**Cell Cycle Analysis of A549 Cells  
Infected With GFP-fused Wild Type  
and Mutant Tankyrase Homologue**

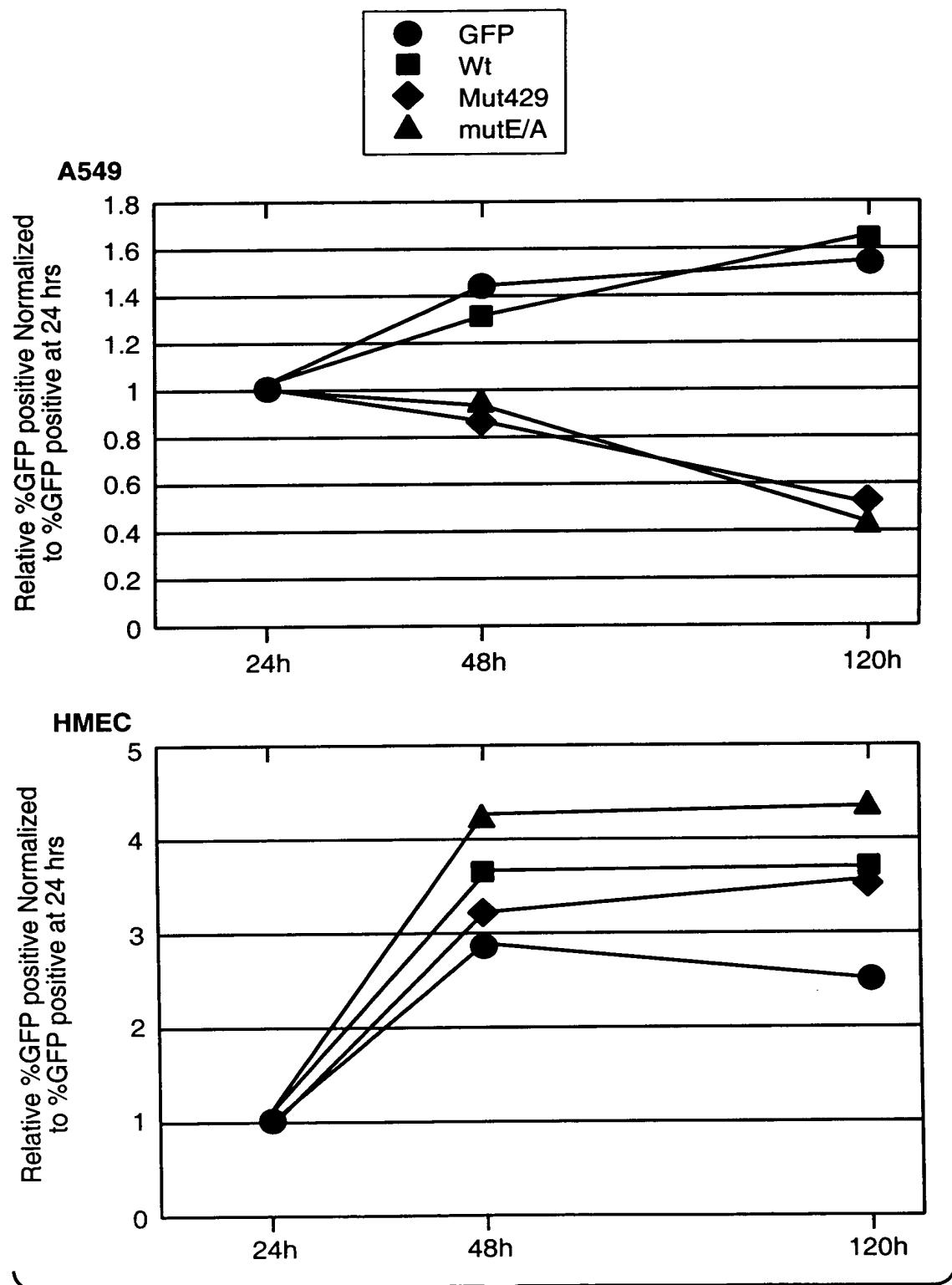


**FIG.\_6A**

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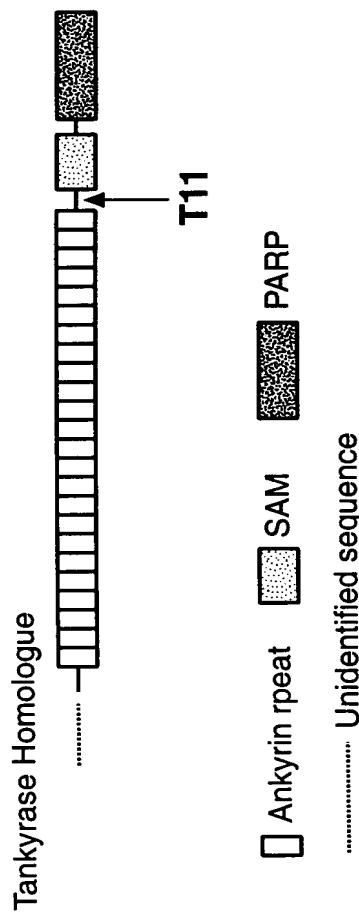
**FIG.\_6B**

**Kinetics of GFP Positive cells in A549 Cells and Human Mammary Epithelial Cells (HMEC) After Retrovirus Infection Encoding GFP-fused Wild Type and Mutant Tankyrase Homologue**



**FIG.\_7**

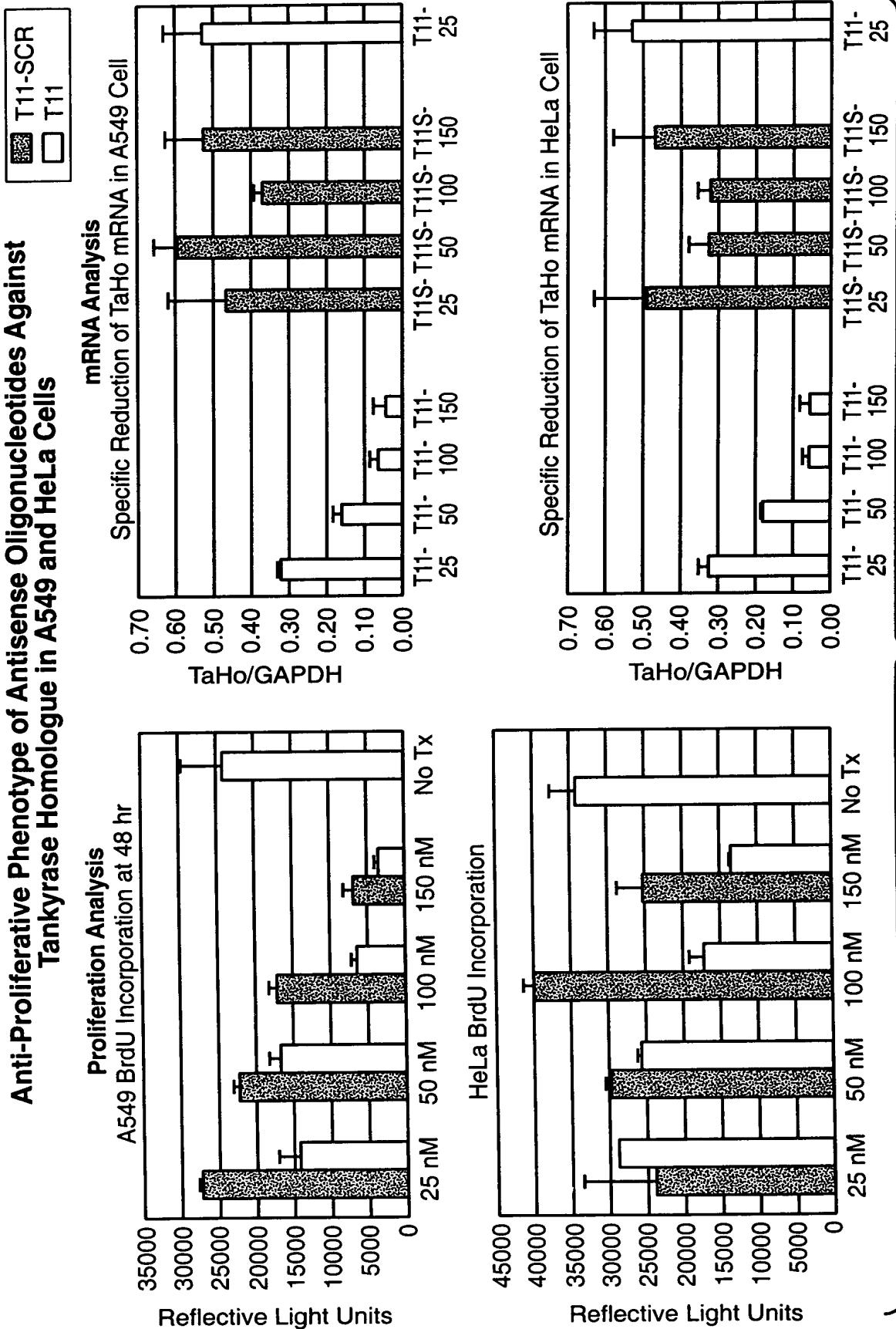
## The Binding Site of Antisense Oligos Against Tankyrase Homologue



T11	Tankyrase Homologue	Tankyrase	GTGGAACAGGGGTGCTCC GTGGAACAGGGGTGCTCCAGTTGGAAAAAGGAGGGTCCAGGAGTAGATTAGCAT ATGCAGGGATGGCGCCGGAAACAGAAAGGAAGGGAAAGTGGTCAGAT	2838 3091
*	*	*	*	*** *** ***

FIG. 8

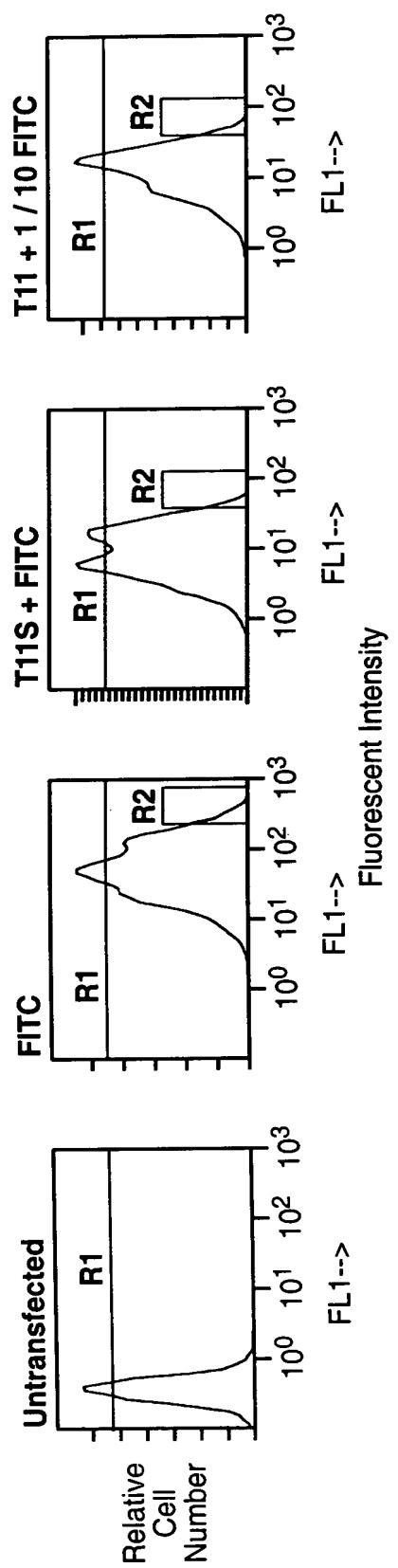
**Anti-Proliferative Phenotype of Antisense Oligonucleotides Against Tankyrase Homologue in A549 and HeLa Cells**



**FIG.-9**

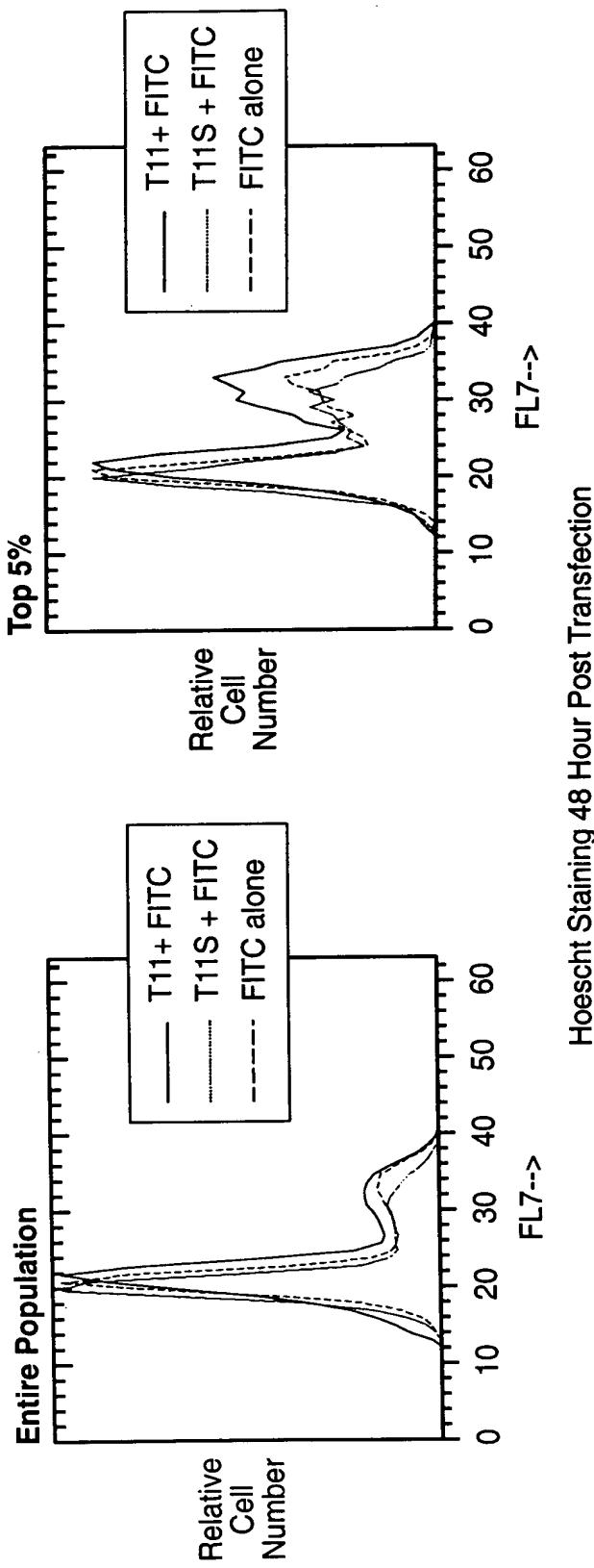
**Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and Control Oligonucleotides (T11S) were transfected with FITC-labeled random 20mer Oligonucleotides (FITC). After 48 Hours, entire population (R1) and Top 5% (R2) of FITC transfected cells were analyzed for cell cycle**

Gates for Cell Cycle Analysis

**FIG.. 10A**

**Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and Control Oligonucleotides (T11S) were transfected with FITC-labeled random 20mer Oligonucleotides (FITC). After 48 Hours, entire population (R1) and Top 5% (R2) of FITC transfected cells were analyzed for cell cycle**

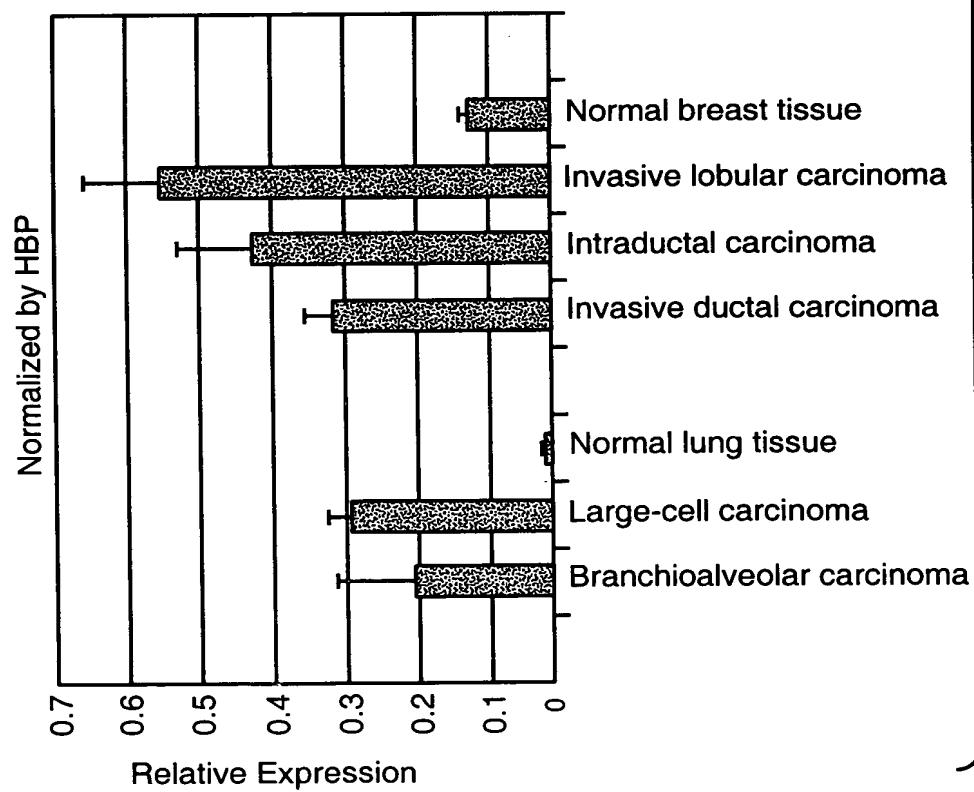
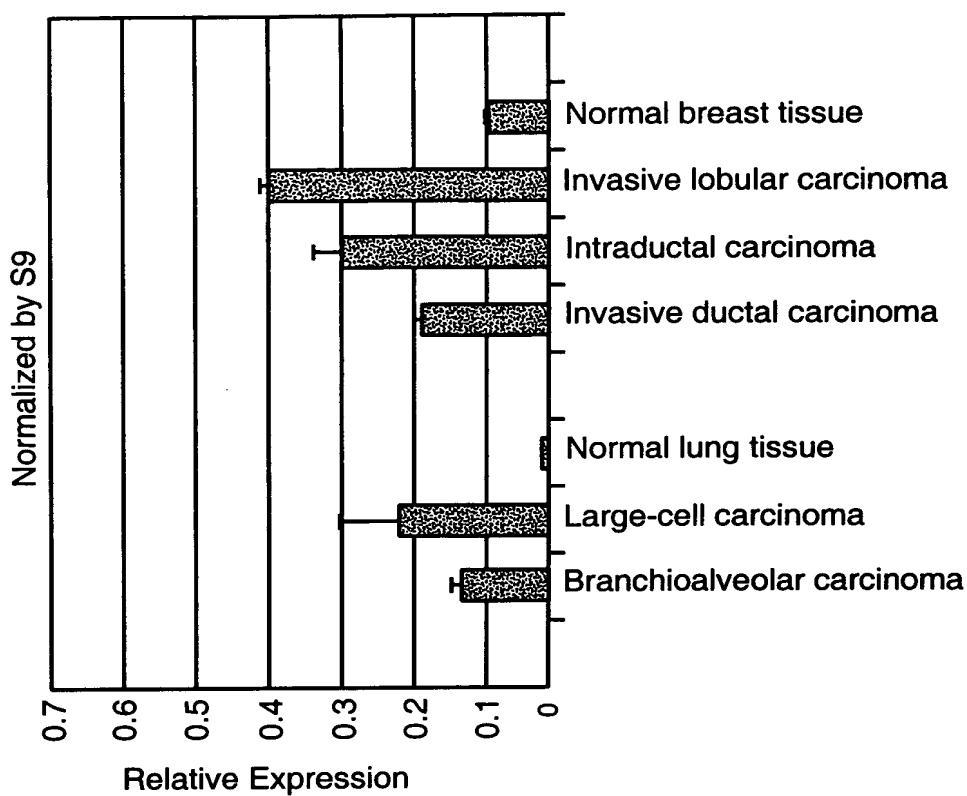
## Cell Cycle Analysis



Hoescht Staining 48 Hour Post Transfection

**FIG.- 10B**

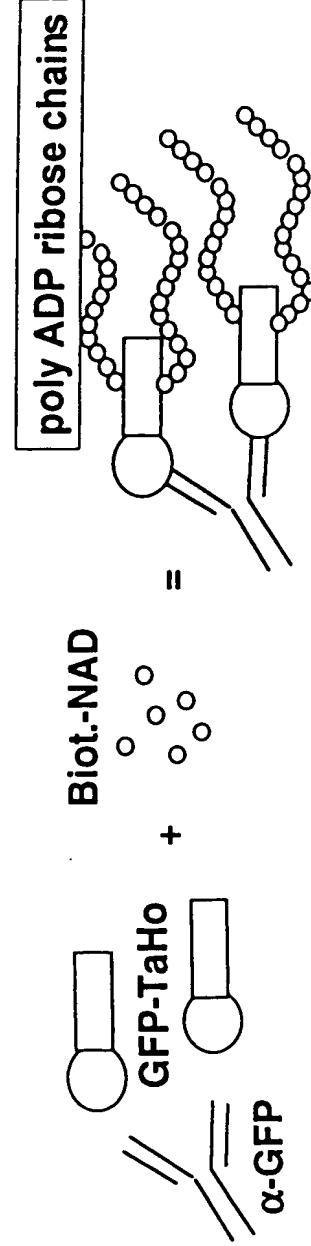
**mRNA Expression of Tankyrase Homologue in Several Tumors and Normal Tissues by a Taqman Analysis, mRNA Expression was Normalized by 90kDa Highly Basic Protein (HBP) and Ribosomal Protein S9 (S9)**



**FIG.- 11**

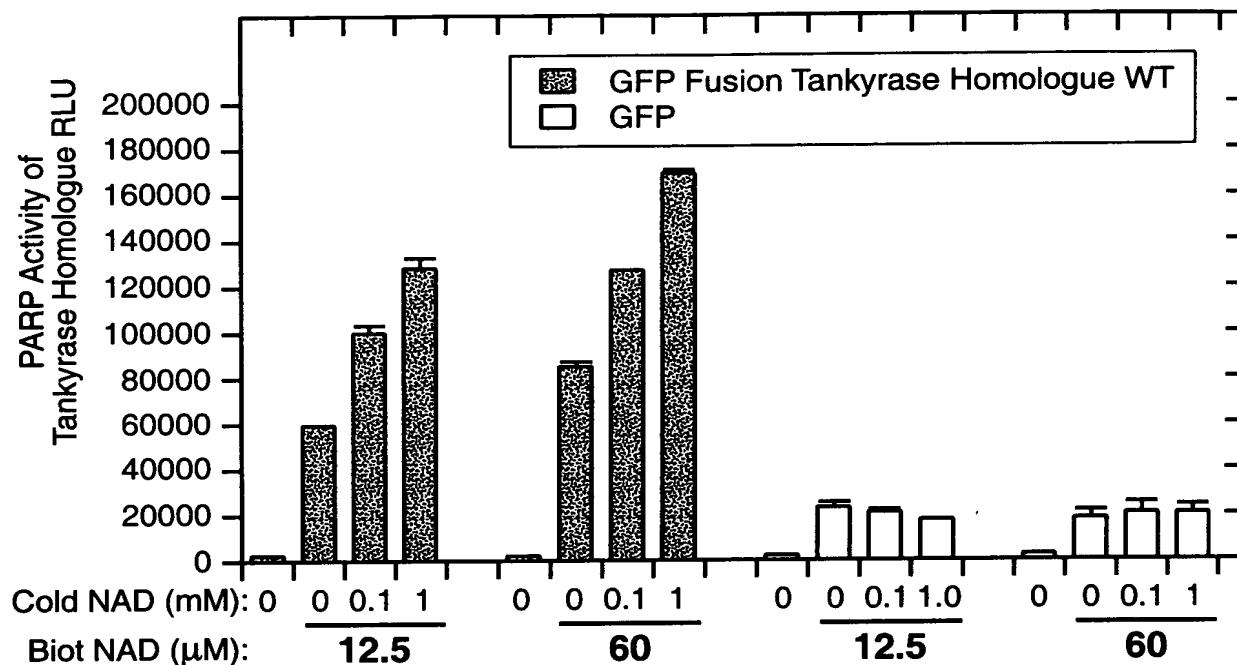
Procedure for Nonisotopic Detection of Poly-ADP Ribosylation  
Using Anti-GFP mAb-Coated Plates

- Protein lysates from 293T cells normalized by GFP fluorescence and total protein →  
Immobilization of GFP-tankyrase homologue in anti-GFP Coated plates →  
Auto PARR reaction with Biotinylated-NAD in 96 wells →  
Detection of poly ADP ribose chains with Streptavidin-HRP and chemiluminescent substrate



**FIG. 12**

**Non-Isotopic Plate-Based Detection of Taho PARP Activity in the Presence of Biotinylated NAD**



**FIG.\_ 13**

**Comparison of IC<sub>50</sub> Values of the PARP Inhibitors**

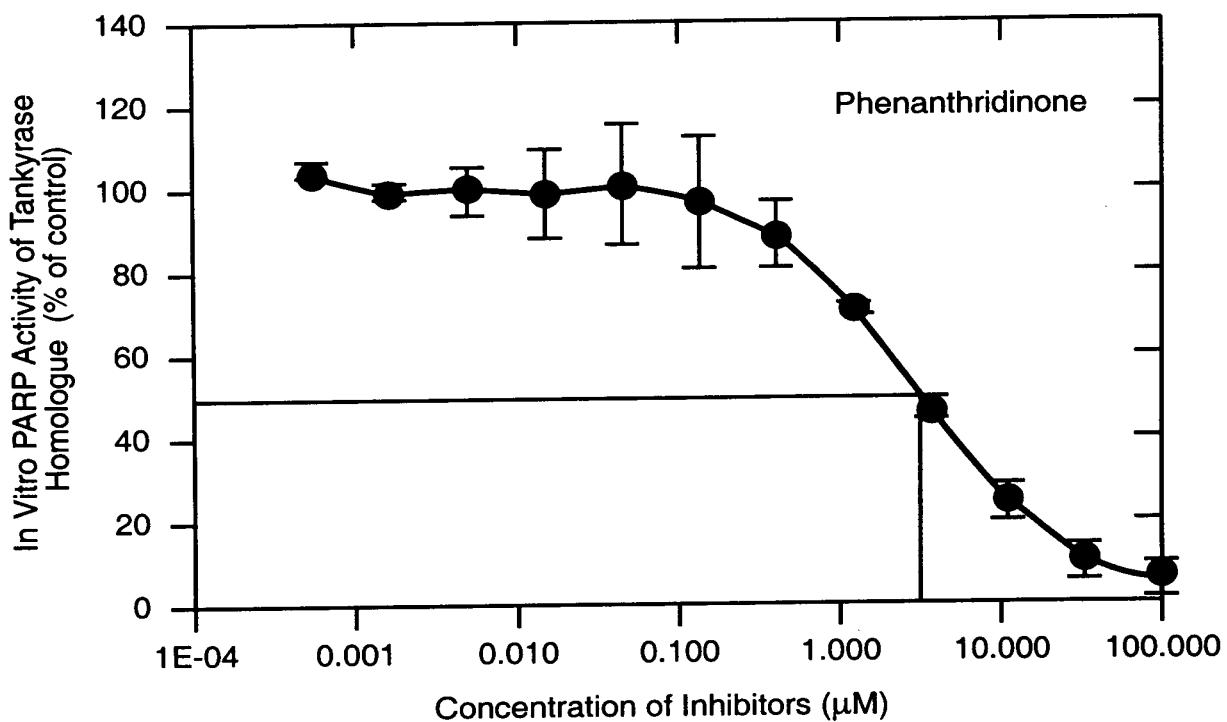
	<u>Approximate IC<sub>50</sub> (nM)</u>	<u>hPARP assay IC<sub>50</sub> (nM)</u>		
		<u>TaHo</u>	<u>Rigel</u>	<u>Decker*</u>
3AB	>50 000		5 000	2 000
6(5H)Phenanthridinone	1 000-2 000		300	
Niacinamide	>50 000		30 000	>>5 000
				31 000

\* Decker P et al., Clinical Cancer Research. 1999 May; 5:1169-1172

\* Rawkin PW et al., J Biol Chem. 1989 Mar 15;264(8):4312-4317

**FIG.\_ 14**

**Inhibition of Tankyrase Homologue PARP Activity  
by hPARP Inhibitors**



**FIG.\_ 15**

**FIG.\_ 16**

*FIG.\_ 16A*

*FIG.\_ 16B*

*FIG.\_ 16C*

**TH-1: Tankyrase Homologue isoform-1, TH-2: Tankyrase Homologue isoform-2  
M (Red): the first methionine in the sequence, Z: stop codon  
In this figure, the first methionine in TH-1 sequence is position 1 (M1)**

## FIG.\_ 16A

Taho C terminus deletion mutant ends at position 429 (K) and adds 28 amino acids because of frame shift.

Taho F/L mutant has the mutation at position 871

Taho E/A dC mutant has the mutation at position 948, ends at position 957 (A) and adds 2 amino acids.

TH-1	-----	-----	-----	-----	-----	-----	-----
TH-2	RCSARRGAAGGQAQRGARVGAAGTAPDPVTAGSQ	-231					
TH-1	-----	-----	-----	-----	-----		
TH-2	AARALSASSPGLLALLAGPGLLRLLLAVAAARIMSGRRCAGGGAACASAEEAVE	-171					
TH-1	-----	-----	-----	-----	*GFGRKDVVVEYLLQNGA	-111	
TH-2	PAARELFEACRNGDVERVKRLVTPKEKVNSRDTAGRKSTPLHFAAGFGRKDVVVEYLLQNGA	-111	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat	
TH-1	-----	-----	-----	-----	-----		
TH-2	SVQARDGGGLIPLHNACSFGHAEVVNLLIRHGADPNARDNWNYTPLHEAAIKGKIDVCIV	-51					
TH-1	NVQARDGGGLIPLHNACSFGHAEVVNLLIRHGADPNARDNWNYTPLHEAAIKGKIDVCIV	-51					
TH-2	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat	Ankyrin repeat
TH-1	-----	-----	-----	-----	-----	•TH1 start	
TH-2	LLQHGAEPITIRNTDGRTALDIADPSAKAVLTGEYKKDELLESARSMSGNEEKMMALLTPLNV	10					
TH-1	LLQHGAEPITIRNTDGRTALDIADPSAKAVLTGEYKKDELLESARSMSGNEEKMMALLTPLNV	10					
TH-2							

***FIG.-16B***

	<u>Ankyrin repeat</u>	<u>Ankyrin repeat</u>
TH-1	NCHASDGRKSTPLHAAAGYNRVKIVQLLIQHGADVHAKDKGDLVPLHNACSYGHYEVTEL Ankyrin repeat	70
TH-2	NCHASDGRKSTPLHAAAGYNRVKIVQLLIQHGADVHAKDKGDLVPLHNACSYGHYEVTEL Ankyrin repeat	70
TH-1	LVKHGACVNAMDWLQFTTPLHEASKNRVECSLLLISYGADPTLNCHNKSAILDAPTPQL LV	130
TH-2	<u>Ankyrin repeat</u>	
TH-1	KERIAYEFFKHSLLQAAAREADVTTRIKKKLSEMVNFKHPOTHEATALHCAAAASPYPKRKQI Ankyrin repeat	190
TH-1	CELLLRKGANINEKTKEFLTPLHVASEKAHNDVVVVVKHEAKVNALDNLGOTSLHRAAY Ankyrin repeat	250
TH-1	CGHIQTCRLLSISYGCDPNIISLOGFTALQMGNEVQOLLQEGISLGNSEADRQLLEAAKA Ankyrin repeat	310
TH-1	GDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVEYLLQHGADVHAKDKGGGLVP Ankyrin repeat	370
TH-1	LHNACSYGHYEVAEILLVKHGAVVNADLWKFTPLHEAAAKGKYEICKLILLQHGADPTKKN Ankyrin repeat	430
TH-1	GMEILLWILKMEIQIFKICLGMQLCZ RDGNTPLDLVKDGTIDQDLLRGDAALLDAAKKGCLARVKLSSPDNVNCRDTQGRHSTP Ankyrin repeat	490

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**FIG.\_16C**

TH-1	LHLAAGYNNLEVAEYLLQHGA DVAQDKGGGLIPLHNAA SYGHVDVAALLIKYNACVNATD	550	
	Ankyrin repeat		Ankyrin repeat
TH-1	KWAFTPLHEAAQKGRTQLCALLAHGADPTLKNQEGQTPLDLVSADDVSALLTAMPSSA	610	
	Ankyrin repeat		Ankyrin repeat
TH-1	LPSCYKPVQVLNGVRSPGATA DALSSGPSSPSSLSAASSLDNLNSGSFSELSVSSSSGTEG	670	
	Ankyrin repeat		Ankyrin repeat
TH-1	ASSLEKKE--VPGVDFSITQFVRNLGLEHLM DIFEREQITLDVLVEMGHKELKEIGINAY	730	
	SAM domain		SAM domain
TH-1	GHRHKLIKGVVERLISGQQGLNPYLT LNTSGSGTILLIDLSPPDDKEFQSVEEEQM STVREHR	790	
TH-1	DGGHAGGIENRYNILKIQKV CNKWLIVERYTHRKEVSEENHN HANERMLFHGSPPFVNALL	850	
TH-1	HKGFDERHAYIGGMFGAGIYFA ENSSKSQNYYVYGGGGTGC PVHKDRSCYICHRLLLFCR	910	
	• F → L mutation		
	PARP domain		
TH-1	VTLGKSFLQFSAMKMAHSPPGH HSVTGRPSVNGLALA EYVIYRGEOQAYPEYLITYQIMRP	970	
	• E → A		• Deletion.
TH-1	EGMVDG 976		